

## BLOOD CHEMISTRY, CYTOLOGY, AND BODY CONDITION IN ADULT NORTHERN GOSHAWKS (*ACCIPITER GENTILIS*)

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**ABSTRACT.**—A bird's physiological state contributes to its reproductive success and survival, yet few baseline physiological data have been published for wild raptors. Mean levels of protein, cholesterol, calcium, uric acid, aspartate aminotransferase, lactate dehydrogenase, and creatine kinase were measured in 29 Northern Goshawks (*Accipiter gentilis*) during 1998–99. None of these substances were significantly different between males ( $N = 8$ ) and females ( $N = 2$ ). Levels of all substances were highly variable among individual birds and unrelated to the body condition index (mass/wing chord  $\times$  tail length  $\times$  culmen length). Total white blood cell count estimates and differential white blood cell counts were not significantly different between the sexes. Of the blood cell measures, only the percent of heterophils and lymphocytes, and the ratio of heterophils to lymphocytes (H/L) differed between birds in good body condition and birds in relatively poor body condition. The H/L ratio has recently been proposed as a reliable measure of stress. Thus, variable H/L ratios between groups of wild birds may indicate differences in stress levels and overall health.

**KEY WORDS:** *Northern Goshawk; Accipiter gentilis; blood chemistry; H/L ratio; body condition.*

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### QUÍMICA SANGUÍNEA, CITOLOGÍA, Y CONDICIÓN CORPORAL EN ADULTOS DEL AZOR NORTEÑO (*ACCIPITER GENTILIS*)

**RESUMEN.**—El estado fisiológico de una ave contribuye a su éxito reproductivo y supervivencia, hasta el momento muy pocos datos fisiológicos de línea base han sido publicados para rapaces silvestres. Los niveles medios de proteínas, colesterol, ácido úrico, aminotransferasa aspartato, lactato deshidrogenasa, y creatina quinasa se midieron en 29 azores norteños (*Accipiter gentilis*) durante 1998–99. Ninguna de estas sustancias fue significativamente diferentes entre machos ( $N = 8$ ) y hembras ( $N = 2$ ). Los niveles de todas las sustancias fueron altamente variables entre aves individuales y no estuvieron relacionadas con el índice de condición del cuerpo (masa/cuerda alar  $\times$  longitud de la cola  $\times$  longitud del culmen). El conteo de leucocitos totales estimados y el conteo diferencial de leucocitos, no fueron significativamente diferentes entre sexos. Entre las medidas de las células sanguíneas, únicamente el porcentaje de heterofilos y linfocitos, y la razón de heterofilos a linfocitos (H/L) difirió entre aves con buena condición corporal y aves en condición corporal relativamente pobre. La razón H/L ha sido propuesta recientemente como una medida confiable de estrés. Así, las razones H/L variables entre grupos de aves silvestres pueden indicar diferencias en los niveles de estrés y en la salud global.

[Traducción de César Márquez]

Northern Goshawks (*Accipiter gentilis*) are found throughout forested portions of the intermountain west (Squires and Reynolds 1997) where they are classified as a “sensitive species” by the United States Forest Service (Beals and Harris 1996, Squires and Reynolds 1997) and a “species of spe-

cial concern” by the Idaho Department of Fish and Game (Beals and Harris 1996). Basic knowledge of adult animal health, including blood-chemistry information, is needed for the management of vulnerable species (Ferrer and Dobado-Berrios 1998), such as the goshawk. However, past research on free-ranging goshawk life history or management does not include an assessment of adult health from blood chemistry or cytology (blood cell) measures. These measures

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provide information regarding nutritional and immunological status, and help us understand ecological and behavioral problems (Ferrer 1990, Stein et al. 1998). Two objectives were addressed in this study: (1) determine baseline means and ranges of blood chemistry and cytology levels in adult breeding goshawks, and (2) determine the relationships among blood chemistry, blood cytology, and body condition.

Blood chemistry is an indirect method of assessing health as blood acts as a means to mobilize and transport nutrients, metabolic products, immune cells, and hormones (Brown 1996). Metabolic substances present in blood plasma reflect avian nutritional health and overall condition (Snyder and Terry 1986, Mauro 1987, Ferrer 1993).

Much like plasma metabolites, plasma enzymes also reflect avian health. Enzymes such as aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine kinase (CK) help regulate metabolic reactions and respond to changes in the body (Mauro 1987). Interpretation of plasma enzyme levels is complicated by natural variation unless levels are significantly higher or lower than reference samples (Ivins et al. 1986, Campbell 1995).

Differential white blood cell counts provide information on the activity level of a bird's immune system and stress response (Dhabhar et al. 1996). Heterophils and lymphocytes are the predominant white blood cells found in raptors (Lavin et al. 1992, Stein et al. 1998). The ratio of heterophils to lymphocytes (H/L) has proven to be a reliable measure of stress in poultry and wild birds and a more accurate measure of stress than individual cell numbers or corticosterone levels (Gross and Siegel 1983, Maxwell 1993, Vleck et al. 2000).

Finally, morphological characteristics measured to provide a body condition estimate can be indicative of individual health (Brown 1996). Body mass is relative to structural size (i.e., wing, tail, culmen length), which is defined as the nutrient-reserve independent size of an individual (Piersma and Davidson 1991). Birds in good condition should have a relatively high ratio of mass to structural size (Brown 1996). We hypothesized that goshawks assessed to be in good body condition by this index would exhibit relatively high levels of blood chemistry measures (e.g., protein, calcium) indicative of adequate nutrition and low levels of cytology measures (e.g., H/L ratio) indicative of an activated immune system, high stress, or disease.

#### MATERIALS AND METHODS

This study was conducted from 1998–99 in the west-central mountains of Idaho. The study area encompassed parts of the Boise, Payette, Snake, and Salmon River drainages where elevations range from 460–3050 m (Steele et al. 1981). Public and private lands were surveyed for goshawks in Washington, Adams, Idaho, Valley, and Boise counties. The study area is a mix of lowland meadows and rangeland and higher elevation mixed coniferous forests. A more complete description of the vegetation and climatic conditions can be found in Hanauska-Brown (2000).

We trapped adult goshawks with a live Great Horned Owl (*Bubo virginianus*) lure to elicit aggressive responses from nesting goshawks and capture them in a dho-gaza net (Detrich and Woodbridge 1994). Upon capturing one adult from a nest, we reset the dho-gaza net in an attempt to catch the other adult.

We collected up to 500  $\mu$ l of whole blood after puncturing the brachial vein with a sterile lancet. Plasma was sent to IDEXX Veterinary Services Laboratory, Inc. (Portland, OR) which measured protein, cholesterol, calcium, uric acid, and three blood enzymes: AST, CK, and LDH. Additional substances were measured in fewer than 10 individuals: albumin, globulin, phosphorous, potassium, sodium, amylase, and alkaline phosphatase. Descriptive statistics only are presented for these variables as statistical analyses were deemed inappropriate.

We evaluated adjusted Sidak *P*-values (Zar 1996) from multiple pairwise comparisons (MULTTEST, SAS Release 6.12, 1996) to determine significant differences between samples collected from females and males, with  $N \geq 8$  samples for each sex (Zar 1996). Adjusted Sidak *P*-values are slightly less conservative than the Bonferroni test and control for comparison-wise error (Zar 1996). We performed Spearman correlation analyses of metabolic substances and enzymes when  $N \geq 8$  individuals for each sex. Assumptions of normality and heterogeneity were tested prior to statistical comparisons, and correlations and nonparametric tests were performed when assumptions were violated.

A blood smear was made for each bird and sent to IDEXX Veterinary Services Laboratory, Inc. The laboratory estimated total white blood cell numbers and percentages of cell types including: heterophils, eosinophils, monocytes, and lymphocytes. We used multiple pairwise comparisons and Sidak *P*-values again to evaluate mean differences between the sexes. We also performed Spearman correlation analyses for all cytological measures.

For the calculation of body condition, we measured mass, unflattened wing chord length, tail length, and culmen length. Wing chord length was measured to the tip of the longest primary, tail length to the tip of the longest rectrix, and culmen length the distance between bill tip and anterior end of the cere. The mass:structural size ratio was determined by dividing mass (g) by the product of wing chord length (mm), tail length (mm), and culmen length (mm).

Preliminary results from our data demonstrated blood metabolite and enzyme measures ( $N = 7$ ) were significantly correlated, as were white blood cell measures ( $N = 6$ ). Thus, we predicted that a suite of blood chemistry and cytology measures, rather than any individual mea-

Table 1. Metabolites and metabolic enzymes measured in Northern Goshawks in west-central Idaho during the breeding seasons of 1998 and 1999 along with ranges reported for 24 captive adult female and male goshawks (Benyon et al. 1996).

SUBSTANCE	FEMALES			MALES			BENYON ET AL. (1996)
	N	MEAN (SE)	RANGE	N	MEAN (SE)	RANGE	RANGE
Total protein (g/dL)	21	1.8 (0.2)	0.9–3.4	8	2.3 (0.2)	1.3–3.2	2.6–4.2
Cholesterol (mg/dL)	21	155.1 (14.8)	33.0–286.0	8	201.0	125.0–282.0	154.7–444.7
Uric acid (mg/dL)	21	12.6 (2.3)	1.8–40.2	8	20.9 (6.6)	9.4–63.0	8.6–14.4
Calcium (mg/dL)	21	4.7 (0.5)	1.8–9.5	8	5.9 (0.7)	3.3–8.0	8.6–10.9
Phosphorous (mg/dL)	4	3.7 (0.7)	2.4–5.0	4	2.2 (0.5)	1.2–3.7	NA
Potassium (mEq/L)	5	3.0 (0.3)	2.5–4.0	4	2.5 (0.3)	1.9–3.1	NA
Sodium (mEq/L)	5	158.6 (7.4)	136.0–181.0	4	159.8 (2.3)	155.0–165.0	NA
A/G ratio <sup>a</sup>	4	0.7 (0.1)	0.5–1.0	4	0.7 (0.03)	0.6–0.7	NA
Globulin (g/dL)	4	1.6 (0.2)	1.2–2.2	4	1.6 (0.1)	1.4–1.8	NA
Albumin (g/dL)	4	1.1 (0.1)	0.9–1.2	4	1.0 (0.1)	0.9–1.2	NA
AST (IU/L) <sup>b</sup>	21	286.2 (26.2)	108.0–561.0	8	375.5	199.0–556.0	176.0–409.0
LDH (IU/L) <sup>c</sup>	21	347.6 (47.9)	136.0–840.0	8	616.4	322.0–1040.0	120.0–906.0
CK (IU/L) <sup>d</sup>	21	127.0 (12.3)	56.0–285.0	8	160.6	56.0–412.0	218.0–775.0
Amylase (IU/L)	4	900.5 (44.1)	790.0–1006.0	4	864.0	567.0–1195.0	NA
Alk. phos. <sup>e</sup> (IU/L)	4	20.5 (4.7)	9.0–31.0	4	20.5 (4.8)	15.0–35.0	NA

<sup>a</sup> A/G = albumin/globulin ratio.

<sup>b</sup> AST = aminotransferase.

<sup>c</sup> LDH = L-lactate dehydrogenase.

<sup>d</sup> CK = creatine kinase.

<sup>e</sup> Alk. phos. = alkaline phosphatase.

sure, would best represent goshawk physiological health. We hypothesized that goshawks with higher body condition indices would exhibit a more healthy physiological profile compared to birds in relatively poor body condition. To test this hypothesis we reduced the physiological data from each bird to an individual factor score using principle components analysis (StatSoft 1998). The factor score was a multi-dimensional physiological representation of goshawk health. In this analysis, we used the factor scores associated with the first eigenvector. Subsequently, factor scores for the 10 goshawks in the best and the worst body condition (as indicated by body condition index) were evaluated using a nonparametric two-sample comparison test (Mann-Whitney *U*-test; Bailey 1995). We used the body condition index as the measure of overall condition as it was independent of the other physiological measures (Hanuska-Brown 2000) and has been used to represent health in other studies (Rising and Somers 1989, Brown 1996). We combined sexes in the analysis because there were no differences in blood chemistry, cytology, or body condition means. Two-sample comparisons were performed on factor scores derived from the entire suite of physiology variables ( $N = 13$ ), the combined metabolic and enzyme variables ( $N = 7$ ), and the suite of blood cell variables ( $N = 6$ ). For significant two-sample comparisons, factor loadings from the principle components analysis were used to help determine which variables had greatest influence on the factor scores (StatSoft 1998). Factor loadings can be interpreted as the correlations between factors (i.e., the set of factor scores

for all birds) and the individual physiological variables (StatSoft 1998). Thus, the magnitude and sign of factor loadings can be used to determine the relative importance of individual variables in explaining the two-sample comparison results.

## RESULTS

Metabolite, enzyme, and white blood cell concentrations were measured in 21 adult female and 8 adult male goshawks (Tables 1–2). There were no between-sex differences in metabolite or enzyme levels ( $P > 0.50$ ). Data on metabolic substances and enzymes from 24 captive adult goshawks (Benyon et al. 1996) are provided for comparison with our data (Tables 1–2). There were no between-sex differences in total white blood cell estimate, individual cell type, or H/L ratio for goshawks measured in this study ( $P > 0.45$ ); however, we note that the differences between female and male monocyte numbers approached significance ( $t = -3.10$ ,  $P = 0.07$ ). The correlation analysis of all blood chemistry variables showed that all metabolites and enzymes were correlated ( $P \leq 0.05$ ) with the exception of the enzyme CK. Correlation analysis of the blood cell

Table 2. White blood cell total estimates ( $\times 10^9/1$ ) and white blood cell types (%) measured in Northern Goshawks in west-central Idaho along with ranges reported for 43 captive adult female and male goshawks (Benyon et al. 1996).

WBC	FEMALES			MALES			BENYON ET AL. (1996)
	<i>N</i>	MEAN (SE)	RANGE	<i>N</i>	MEAN (SE)	RANGE	RANGE
Total estimate	21	9.8 (1.0)	4.0–20.0	8	8.7 (1.2)	4.0–13.0	4.0–11.0
Monocytes	21	2.3 (0.4)	0.0–6.0	8	5.3 (1.3)	0.0–12.0	1.0
Lymphocytes	21	38.2 (2.5)	16.0–58.0	8	46.8 (5.0)	26.0–66.0	14.0–19.0
Eosinophils	21	19.6 (1.4)	6.0–33.0	8	13.6 (2.3)	4.0–23.0	7.0
Heterophils	21	40.0 (2.4)	19.0–58.0	8	34.3 (5.0)	12.0–54.0	36.0–65.0
H/L ratio <sup>a</sup>	21	1.27 (0.17)	0.37–3.31	8	0.89 (0.23)	0.18–2.00	NA

<sup>a</sup> Heterophils to lymphocytes.

counts and white blood cell estimates demonstrated significant patterns ( $P \leq 0.05$ ) among several variables. The mean body condition ratio was  $4.39 \pm 0.08$  (range = 3.81–4.96) for females and  $4.90 \pm 0.03$  (range = 3.86–6.16) for males (Table 3). There was no significant difference between female and male body condition ratios (Wilcoxon signed rank,  $z = 1.51$ ,  $P = 0.13$ ).

The two-sample comparison test using all physiological variables ( $N = 13$ ) for the 10 goshawks with the highest and lowest body condition scores detected no difference ( $N = 20$ ,  $U = 27$ ,  $P = 0.25$ ). Similarly, the comparison using the combined metabolite and enzyme variables ( $N = 7$ ) showed no difference between goshawks of different body condition ( $N = 20$ ,  $U = 36$ ,  $P = 0.29$ ). Two-sample comparison tests on factor scores derived from blood cell variables ( $N = 6$ ), however, showed a difference between good and poor body condition goshawks ( $N = 20$ ,  $U = 3$ ,  $P < 0.01$ ). Factor loadings for each variable indicated that the H/L ratio (negative loading) was the variable primarily responsible for the observed difference. These results are consistent with paired comparisons performed on individual variables that demonstrated high lymphocyte numbers, low heterophil num-

bers, and low H/L ratios for goshawks in good condition (Hanauska-Brown 2000). There was a significant negative correlation between H/L and body condition ( $N = 30$ ,  $r^2 = -0.48$ ,  $P < 0.01$ ).

#### DISCUSSION

Concerns over the population status of goshawks throughout the intermountain west region (Kennedy 1997) prompted us to seek a means of indexing the health of adult breeding goshawks. We based this index on morphological measurements, blood chemistry, and cytological characteristics. We focused on these variables because other researchers have questioned the assessment of population viability using occurrence, density, or annual productivity estimates (e.g., Van Horne 1983, Franklin et al. 2000).

Our data represent baseline metabolite, enzyme, and blood cytology levels for a sample of free-ranging goshawks in central Idaho. The only other baseline data available for goshawks are from "normal, healthy" male and female goshawks of various ages, housed in rehabilitation centers (Benyon et al. 1996). Captive birds are provided with a continuous food source, but also experience a multitude of stressors (e.g., confinement and handling) not

Table 3. Morphology measures from Northern Goshawks in west-central Idaho.

MORPHOLOGY	FEMALES			MALES		
	<i>N</i>	MEAN (SE)	RANGE	<i>N</i>	MEAN (SE)	RANGE
Mass (g)	21	1006.8 (14.8)	870–1134	8	766.9 (15.7)	710–824
Wing (mm)	21	353.8 (1.7)	341–372	8	325.4 (3.8)	310–337
Tail (mm)	21	261.7 (1.2)	249–272	8	225.1 (2.4)	212–232
Culmen (mm)	21	24.8 (0.3)	23.2–29.9	8	21.1 (0.3)	19.7–25.1

present in the wild (Stein et al. 1998). Captive birds receive a steady, high protein diet (Gee et al. 1981, Garcia-Rodriguez et al. 1987) that provides an adequate supply of amino acids for the synthesis of blood proteins (Ferrer et al. 1987). Indeed, total protein was higher for the captive goshawks than for the birds sampled in this study. Wild goshawks may experience periods of fasting (Newton 1979) and may be forced to specialize on certain prey species (Newton 1979, Younk 1996) leading to a mineral or dietary deficiency (Snyder and Terry 1986). Birds in the wild engage in activities (e.g., courtship, territoriality, nestling care) that reduce the time available for foraging, which also may lead to dietary deficiencies, particularly in poor-quality habitat.

Calcium levels were lower in wild than in captive goshawks (Benyon et al. 1996), which also can be attributed to dietary differences. Captive birds receiving chicken carcasses, rabbits, and other 'whole' food items (Gee et al. 1981) obtain calcium from ingesting bones and other body parts. Conversely, captive birds fed only red meat (including muscle, heart, and liver) were found to be deficient in calcium (Graham and Halliwell 1986). Dietary calcium in the wild comes from a varied diet including whole prey items (Graham and Halliwell 1986, Squires and Reynolds 1997). However, wild birds may not be able to capture a steady diet of suitable prey, and low levels of calcium may reflect fasting periods (Halliwell 1981).

The mean uric acid level for females was within the range reported for captive goshawks, but the mean for males was above the reported range (Benyon et al. 1996) by more than 6 mg/dL. Uric acid, a nitrogenous waste product, is typically elevated in birds with high protein diets (Bell and Sturkie 1965, Gee et al. 1981). High uric acid levels in free-ranging birds can also be attributed to decreased body condition or food stress (Handrich et al. 1993, Balbontín and Ferrer 2002, Casado et al. 2002). Males are under high energy demands during the breeding season as they hunt for the adult female and young, as well as for themselves. Mean cholesterol levels from sampled birds were within the range of cholesterol values reported for captive goshawks (Benyon et al. 1996).

The three enzymes we measured are quite variable in captive goshawks (Benyon et al. 1996). Such individual differences are common in avian species due to natural variation in habitat, genetics, body condition, environmental influences, and

other factors (Gee et al. 1981, Hoffman et al. 1985, Stein et al. 1998). For example, Stein et al. (1998) documented more variability in wild populations of American Kestrels (*Falco sparverius*) and Red-tailed Hawks (*Buteo jamaicensis*) than in captive birds. Mean levels of AST and LDH measured in this study were within the wide range of enzyme levels in captive goshawks (Benyon et al. 1996). However, lower levels of CK were measured in wild versus captive birds (Table 1). Average or low CK levels can indicate enhanced muscle strength and endurance (Apple and McGue 1983, Knuth and Chaplin 1994). For example, flight training in captive Red-tailed Hawks improved the structural integrity of muscles and lowered plasma CK levels (Knuth and Chaplin 1994). The low levels of CK observed in this study may reflect increased muscle strength and flight endurance (Apple and McGue 1983, Knuth and Chaplin 1994) in the wild birds.

Individual variation due to proximate factors may play a major role in variable blood chemistry measures, but many other influences can affect the measurement of health in wild birds. Garcia-Rodriguez et al. (1987) demonstrated diurnal and circadian rhythms of calcium, uric acid, and cholesterol levels in raptors. We could not control the time of day of sample collection because of the logistical constraints of trapping breeding goshawks. Time of sample collection in this study ranged from 0700–1900 H. Variation in the levels of protein, uric acid, calcium, and cholesterol in individual goshawks in this study, therefore, may be attributed to recent feeding, fasting periods, or circadian and diurnal rhythms.

Geographic location also may affect blood chemistry measures. Soil types and environmental contamination affect physiology, as raptors incorporate locally-obtained nutrients and toxins from their prey items (Hoffman et al. 1985, Ferrer and Dobado-Berrios 1998). Differences in total protein, uric acid, cholesterol, and calcium were found between two populations of Adalbert's Eagle (*Aquila adalberti*) foraging in different regions of Spain (Ferrer and Dobado-Berrios 1998). Qualitative differences in each population's diet were suggested as the cause of the physiological differences (Ferrer and Dobado-Berrios 1998). Similarly, differences in prey abundance, prey availability, and habitat in central Idaho may be reflected in goshawk blood chemistry measures. For example, goshawks in the western region of the study area likely consumed more ground squirrels (*Spermophilus* spp.)

than goshawks in the northern regions, which probably consumed more birds (pers. observ.).

Goshawk samples from this study showed increased numbers of lymphocytes, monocytes, and eosinophils compared to the ranges reported for captive goshawks (Benyon et al. 1996). Increased levels of particular leukocytes or white blood cell total estimates can indicate physiological stress or decreased immunocompetence (Smith and Bush 1978, Kontecka et al. 1999). A shift from normal values in any direction can indicate disease (Campbell and Dein 1984). Blood loss or tissue damage at the collection site can also affect white blood cell counts. Our blood collection method may have overestimated circulating white blood cells because hemorrhage induced by puncturing the wing vein produces tissue damage that triggers clotting and attracts white blood cells. Capillary blood in humans (e.g., blood collected into capillary tubes after finger pricks) has higher white blood cell counts than blood collected intravenously (Dae et al. 1988, Kayiran et al. 2003). We minimized this potential change in white blood cells by collecting blood immediately upon puncturing the vein.

The H/L ratio was significantly different between birds in relatively good, compared to birds in relatively poor, condition. Such differences suggest decreased immunocompetence or higher rates of stress in birds of poor condition. The H/L ratio has been shown to be a less variable indicator of stress than differential counts and more reliable than corticosterone levels in reflecting chronic stress (Vleck 2002).

One of the main objectives of this work was to explore the relationships between a noninvasive body condition index and the blood chemistry and cytology of wild breeding goshawks. We expected less variability in the blood parameters we observed and stronger relationships among the various physiological measurements and body condition than we detected. Our small sample size undoubtedly influenced variability in our measures, and some of the assumptions underlying the mass/length technique we used to assess body condition have recently been questioned (Green 2001). Furthermore, considering that all birds sampled were breeding, our "poor" condition birds may not have been in poor condition relative to the overall wild population. Despite these concerns, our finding that the relative differences in body condition among breeding birds were reflected in the H/L

ratio highlights the potential sensitivity and utility of this technique.

Our results also illustrate the difficulty in evaluating the physiological state of a bird at any given time. Metabolite and enzyme levels appear to be influenced by too many external and individual factors to serve as accurate barometers of individual health. Despite the limitations inherent in physiological studies of wild raptors such as ours, the H/L ratio and body condition index hold promise as indicators of adult goshawk health. Further study of free-living raptor physiology is warranted to assess the value of the H/L ratio as a measure of individual health.

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